

potential is generated by providing a concentration gradient of a charged substance inside and outside of liposomes and a drug which can be ionized is encapsulated in the liposomes due to a pH gradient or a  $\text{Na}^+/\text{K}^+$  concentration gradient to thereby inhibit the leakage of a drug from the liposomes. As a method of encapsulating a drug in liposomes and inhibiting the leakage thereof similarly using a pH gradient, Barenholz et al. have invented a method using a pH gradient inside and outside of liposomes which is achieved by an ammonium ion gradient using ammonium sulfate (Japanese Patent No. 2,659,136). Both of these methods are not restricted in the particle size of the liposomes to be used, and these liposomes involve small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), multilamellar vesicles (MLVs) and the like. On the other hand, Maurer et al. reported that when ciprofloxacin was encapsulated in LUVs of 190 nm in an average particle size by the method under a pH gradient using ammonium sulfate, ciprofloxacin rapidly leaked out of the LUVs in 50% mouse serum at 37°C (*Biochim. Biophys. Acta*, 1374, 9 (1998)). According to this report, ciprofloxacin was not crystallized (precipitated) in the liposomes, different from doxorubicin or the like, and thus leaked out. Thus, the methods presented by the two patents as described above are not necessarily the most desirable methods from the viewpoint of the leakage of drugs encapsulated in liposomes. Therefore, further improvement has been required.